Mechanisms of Animal Navigation in Odor Plumes

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Abstract. Chemical signals mediate many of life's processes. For organisms that use these signals to orient and navigate in their environment, where and when these cues are encountered is crucial in determining behavioral responses. In air and water, fluid mechanics impinge directly upon the distribution of odorous molecules in time and space. Animals frequently employ behavioral mechanisms that allow them to take advantage of both chemical and fluid dynamic information in order to move toward the source. In turbulent plumes, where odor is patchily distributed, animals are exposed to a highly intermittent signal. The most detailed studies that have attempted to measure fluid dynamic conditions, odor plume structure, and resultant orientation behavior have involved moths, crabs, and lobsters. The behavioral mechanisms employed by these organisms are different but generally integrate some form of chemically modulated orientation (chemotaxis) with a visual or mechanical assessment of flow conditions in order to steer up-current or upwind (rheo- or anemo-taxis, respectively). Across-stream turns are another conspicuous feature of odor-modulated tracks of a variety of organisms in different fluid conditions. In some cases, turning is initiated by detection of the lateral edges of a well-defined plume (crabs), whereas in other animals turning appears to be steered according to an internally generated program modulated by odor contacts (moth counterturning). Other organisms such as birds and fish may use similar mechanisms, but the experimental data for these organisms is not yet as convincing. The behavioral strategies employed by a variety of animals result in orientation responses that are appropriate for the dispersed, intermittent plumes dictated by the fluid-mechanical conditions in the environments that these different macroscopic organisms inhabit.

Introduction

By and large, life exists in one of two fluid environments, air or water. These media offer a vast array of different conditions, even the most severe of which have been successfully colonized by life. Although the range of conditions that support life appears to be extremely broad, the fact that air and water are fluids means that, in principle, they are governed by the same physical laws (Denny, 1993). Thus the physical behavior of fluids is important to organisms that orient with respect to chemical signals, because fluid dynamics impinge directly upon the distribution of odorant molecules in time and space. Furthermore, the fluid environment also impinges directly upon the locomotory activity of organisms. Hence, fluid conditions dictate both the dispersal of odor molecules in the environment and the ability of organisms to orient to the signal being transported. The physical nature of air and water with particular reference to biological systems has been excellently reviewed in considerable detail elsewhere (Denny, 1993; Vogel, 1994; Weissburg, 1997, 2000), so the brief discourse here will serve as the framework for the more substantive treatment that will be given to the mechanisms employed by animals to navigate and orient with respect to chemical signals. The focus will be on macroscopic animals, although examples of smaller life-forms will be used where appropriate.

The ability to sense and respond to the chemicals present in the environment exists in virtually all living creatures from unicellular bacteria to complex, multicellular organisms. The scale of these organisms covers many orders of magnitude, from the smallest single cell to the largest creatures that exist today. Biological as well as physical limitations must, therefore, have shaped the types of chemical signals that a particular organism can detect and respond to effectively.

What is an Odor Plume, or How do Fluid Physics Affect the Distribution of Odor Molecules in Time and Space?

When molecules escape a solid substrate they become subject to physical forces in the fluid. What features of fluids are important in determining how molecules are dispersed once those molecules become fluid-borne? In a fluid that is not moving, molecules that escape the substrate form a concentration gradient that is highest closest to the source and lowest at points farthest away. Obviously, the molecular weight of a molecule has a significant impact on the time taken for a concentration gradient to be established throughout a given volume of fluid. Where molecular diffusion is the main force driving the distribution of molecules, the only signal available to an animal is the chemical itself and its spatiotemporal gradient.

For organisms of our scale it seems hard to envisage situations in which fluids are completely static and molecular diffusion is the predominant force responsible for distributing molecules. However, many organisms exist at scales where molecular diffusion is the salient force behind the distribution of chemical signals. Take a bacterium, for example, that exists within a small volume of fluid. Diffusion will distribute molecules emitted by a source, allowing a concentration gradient to form and orientation with respect to that gradient to occur. Nevertheless, for most macroscopic organisms, fluid movement has a major effect upon the dispersion of chemical signals. If we imagine a simple situation in which a fluid is flowing smoothly over a source of chemical molecules, the fluid is actually static at the point of contact with the substrate. The diffusive sublayer is the region closest to the substrate, where fluid motion comes to rest. This sublayer is one of several that together constitute what is known as the boundary layer (Denny, 1993; Vogel, 1994; Weissburg, 1997, 2000). Across the boundary layer there is a velocity gradient until the fluid reaches 99% of the mean flow velocity (Weissburg, 1997, 2000). Boundary layers have important implications that affect both the distribution and detection of chemical signals, and they are, therefore, of particular importance to animals that use chemical cues to orient. For molecules that pass through the boundary layer, molecular diffusion continues but, depending upon the conditions (such as flow speed of the fluid), the movement of the fluid now plays the major role in distributing molecules in time and space. In smooth, steady-flow environments, the interaction between molecules and flow forms a plume that has relatively distinct boundaries, with a strong gradient across the transverse axis and a weaker longitudinal gradient.

Laminar flow conditions, like molecular diffusion, are relatively uncommon. Anyone who has been fascinated by the patterns of smoke or steam issuing from a chimney and being transported downwind will immediately appreciate the fact that fluid flow is rarely stable. Instead, swirling and eddies within the moving air seem to play a major role in distributing smoke particles. The chaotic movements of the fluid are known as turbulence and are caused by fluctuations in velocity. Turbulence may be caused by, among other things, the flow of fluids around stationary objects (that may or may not be acting as sources of chemical emission) that induce the formation of eddies and vortices in their wake. Various mathematical terms can be used to describe the nature of the fluid environment (*e.g.*, the Reynolds number). A complete description of these terms and their implications for fluid flow can be found in extensive reviews elsewhere (Denny, 1993; Vogel, 1994; Belanger and Willis, 1996; Weissburg, 1997, 2000).

Importantly, particles being transported in turbulent eddies have a tendency to move down a concentration gradient, but turbulent diffusion is a property of how the fluid moves and not of the particles themselves. Therefore, in a plume created by turbulent diffusion, unlike one produced by simple molecular diffusion, the molecular weight of a molecule does not impact its distribution. Consequently, molecules that are released from the substrate at a certain ratio should retain their relative concentrations in situations where turbulent flow dominates. Hence, odor blends could comprise molecules of widely varying molecular weight. This certainly may be the case for odors that are passively released into the environment (those from plants, for example, may be a mixture of compounds of low and high molecular weights). However, odor mixtures that are actively produced and emitted (such as the female-produced pheromonal mixtures of different moth species) are not highly divergent, probably because the biosynthetic pathways responsible for manufacturing the molecules have been well conserved over evolutionary time.

Another attribute of turbulent flow conditions is that the mean direction and speed of fluid flow may be relatively constant, but the instantaneous flow pattern at any point in the plume might be difficult to predict—local flow fields may even be heading in the direction opposite to mean flow. The physical conditions that result in the turbulent dispersal of molecules therefore have several important effects that impinge upon the use of chemical signals by animals for navigation:

1. Particles are distributed intermittently. Small-scale eddies can result in fine-scale intermittency, whereas larger scale intermittency is caused by shifts in the direction of flow and results in plume meandering. The odor patches themselves may contain information that can be used to orient and locate the odor source.

2. Fluid flow properties and their effects upon the distribution of odorous molecules in time and space are potential sources of information for navigation.

3. Once dispatched from a source, odors comprising

more than one molecule are present as a blend and retain their ratios relative to one another, even though there may be disparities in the molecular weights of the constituent molecules.

To produce changes in the behavior of an organism, odorants must be detected by some type of chemosensory structure. Physical constraints similar to those that affect the release of molecules into a fluid are equally important in their detection. Most significantly, because detectors are housed in solid structures around which the moving fluid is displaced, the detector has a boundary layer associated with it that odorous molecules must traverse before their capture by the chemosensory apparatus (Vogel, 1983; Loudon *et al.*, 1994; Koehl, 1996). Boundary layers around olfactory appendages can be affected by the speed with which the organism is moving relative to the fluid or by movements of the appendages themselves through the fluid (*e.g.*, antennule flicking in lobsters. Moore *et al.*, 1991a). Thus, the external morphology of a chemosensory appendage and its position relative to the animal's body (which will experience a different boundary layer) may play an important role in determining the efficiency of the olfactory structure in capturing chemical cues.

Information Available in Odor Plumes

From the brief discussion above it should be clear that several sources of information about the position of an odor source origin relative to the organism in question can potentially be extracted from the odor plume. Of course, this assumes that arrival at or movement away from the odor source is the behavioral objective of the organism in question. Two potential sources of information that pertain to the location of an odor source are discussed:

1. Presence or absence of the odor and any gradients associated therewith

The presence or absence of odor is obviously a crucial feature of any odor plume, and how animals respond to odor or odor loss has helped us understand the mechanisms animals use to navigate. In the absence of flow or visual stimuli associated with the origin of odor emission, the chemical signal itself is the only source of information that can be extracted. In such cases, the ability to detect the chemical gradient can be used to locomote toward or away from the odor source. Mechanisms known to be used for orienting with respect to chemical gradients include chemotaxis or chemokinesis and rely upon the ability to sample the milieu either spatially (comparing inputs to more than one chemosensory structure at the same time and responding to balance inputs—more correctly known as tropotaxis) or temporally (sampling at one location, moving, then sampling again—more correctly known as klinotaxis). Taxes

refer to the ability to direct motion relative to the source of stimulation, *e.g.*, up or down a chemical gradient; kineses are generally reserved for situations in which some measure of locomotory output increases or decreases, but the resulting motion is random with respect to the stimulus source (see Bell and Tobin, 1982, for review of these terms). This latter discussion serves to emphasize that understanding both the locomotory activity of an organism and the strategy for sampling the odor environment are important in accurately describing the behavioral mechanisms utilized.

In turbulent odor plumes the fluid dynamic conditions play a major role in creating the spatiotemporal distribution of odor molecules. Several studies have measured odor plume structure either in water or air to determine odor parameters that have the potential to be extracted and used by animals that navigate in odor plumes (Murlis, 1986, 1997; Murlis *et al.*, 1992; Moore and Atema, 1991; Zimmer-Faust *et al.*, 1995; Finelli *et al.*, 1999). In a laboratory flume, dopamine-sensitive carbon fiber microelectrodes have been used to measure fine-scale odor plume structure (Moore *et al.*, 1989; Moore and Atema, 1991). Other studies have utilized the same electrochemical techniques in combination with fluorometric measurements (the fluorescein dye used also provides a useful visible marker) to characterize odor plume structure under known hydrodynamic conditions in a simple aquatic environment such as an estuarine tidal creek (Zimmer-Faust *et al.*, 1995; Finelli *et al.*, 1999). Measurements at fast temporal time scales show that the odor plume is highly intermittent, with bursts of high odor concentration interspersed with low or zero concentration. In addition, the peaks and onset slopes of odor transients vary systematically along both transverse and longitudinal axes of odor plumes (Moore and Atema, 1991; Zimmer-Faust *et al.*, 1995; Finelli *et al.*, 1999). The lateral edges of these aquatic plumes were represented by a sharp decline in the presence of both the electrochemical tracer and the fluorescein dye over a short distance. This transverse gradient was much greater than any concentration gradient along the longitudinal axis of the plume (Zimmer-Faust *et al.*, 1995). As distance from the source increased, odor plumes tended to spread horizontally and vertically, and mean peak height and slope of odor fluctuations decreased (Moore and Atema, 1991; Zimmer-Faust *et al.*, 1995; Finelli *et al.*, 1999). Nevertheless, much larger fluctuations in concentration than might be predicted from time-averaged plume models were detected far downstream (Moore and Atema, 1991; Zimmer-Faust *et al.*, 1995). Organisms that orient in such plumes may use intermittency or cues derived from the shape of odor pulses to steer in the direction of the odor source (see below; Weissburg, 1997). The fluid dynamic properties of air and terrestrial recording sites result in greater odor-plume intermittency compared to aquatic sites such as an estuarine creek. Indeed, airborne plumes created by an ion source and measured in the field

are highly intermittent (Murlis, 1986, 1997; Murlis *et al.*, 1992), so much so that even in the plume the signal has been estimated to be absent for 60% to 90% of the time (Murlis, 1997).

2. Odor presence or absence coupled with fluid flow information (mechanical or visual)

The presence of flow (either smooth or turbulent) provides an additional source of information that can be integrated with the detection of chemical signals. Because the direction of mean fluid motion is downstream of an odor source, any odor that is detected must have come from upstream. By resolving direction of fluid movement, an organism can determine what direction to take towards a distant and frequently unseen source of odor. The mechanism for the detection of flow depends, to a large degree, upon the life history of the organism in question. For animals that dwell on a fixed substrate, flow can be detected by mechanical means—*i.e.*, pressure differences detected by mechanoreceptors located on the animal's body, head, or other appendages. The direction of flow can be gauged by the deflection of mechanosensors with reference to the substrate. Detection of flow in this manner can be compromised by locomotory activity that will also stimulate mechanoreceptors. For this type of flow detection to operate efficiently, the organism must be able to distinguish movement of the flow due to the flow itself from activity in mechanoreceptive cells resulting from locomotion. In other substrate-bound organisms, flow direction can be estimated by differential cooling: a dog's nose and your own finger, wetted and held in the air, are instruments for detecting wind direction. Animals that orient to odor sources while flying or swimming are faced with a somewhat more complex task. Even though mechanical detectors can be stimulated by the activities of flying or swimming, the need to discriminate between movement of the fluid due to flow and movement of the fluid due to self-propulsion through it means that mechanoreception may not be a particularly reliable source of information for determining flow direction. Vision is far more reliable because locomotion can be referenced to fixed points in the environment such as the ground pattern or riverbed. In fact, experiments with flying moths and other insects have shown the extraordinary degree to which these creatures rely upon visual feedback in making their way upwind in an odor plume (Kennedy, 1939; David, 1986; Colvin *et al.*, 1989).

Biological Limitations

In addition to the constraints imposed by the physics of the fluid medium in which an organism exists, many biological constraints must be included in any understanding of how animals navigate and orient with respect to odor plumes. These biological restrictions can be broken into several categories that can be generally applied to all organisms, no matter what their size and scale:

Transduction

The first limitation concerns the speed with which chemical signals can be transduced by a chemosensory system. The ability to quickly process information arriving at the periphery is essential because it affects the entire response of the organism. For example, how frequently can the milieu be sampled and how well can sequentially arriving pulses be resolved (*i.e.*, how close can two pulses be without being perceived as a single pulse)? These sampling parameters may be particularly important for most macroscopic organisms whose olfactory environment includes turbulent plumes and intermittency as normal features. The threshold of response may also be an important constraint in responding to chemical signals. A certain number of molecules of the same type may have to arrive simultaneously (or within the integration period of the sensor) to produce a response in the sensory apparatus. Even the simplest systems are limited by the speed with which odorant molecules are cleared from their receptors (Denny, 1993). Molecules that occupy a binding site effectively prevent a receptor from responding to future encounters with odor. Thus rates of sensory adaptation and disadaptation certainly influence the ability of an organism to respond to repeated chemical stimuli. In animals with even simple nervous systems these peripheral processes may have higher order corollaries such as habituation and learning that can affect the behavioral performance.

Processing

The second important biological limitation is how quickly information is processed by the organism. Processing may or may not involve communication with other cells. For example, transduction of chemical signals by a unicellular bacterium in a chemical gradient may not be followed immediately by a response, because other biochemical pathways have to be activated before the cell changes direction or rate of locomotion. Similarly, in organisms with a multicellular nervous system, the existence of chemical connections between neurons imposes an inevitable delay between transduction events at the periphery and the resulting behavioral response. This delay affects the rapidity with which an organism can respond to the chemical environment.

Behavior

Behavioral limitations include constraints imposed by the organism itself. There are obvious gross restrictions which include, for example, only being able to walk or run instead of fly. In addition, there are finer locomotory limitations. In other words, a bacterium can move only so many body

lengths or make directional changes only so many times every second; each species of moth has a maximum sustainable flight speed, etc. Of course, there will be variation among individuals, but each organism has an upper limit. These behavioral effects are not independent of delays caused by the transduction and processing events mentioned above but are inclusive of them; together they can be considered as a behavioral latency. Taken together, these biological constraints influence the speed with which organisms perceive and react to changes in the chemical environment. Our own ability to notice such changes is constrained by our ability to resolve differences in the behavioral output of organisms. Marked changes can be readily observed, but more subtle variations in behavior may reflect important fine-tuning of locomotory activity that might easily be overlooked with observational techniques that do not sample the behavior at a sufficiently fast frequency.

## Mechanisms of Navigation in Odor Plumes

One cannot help but notice that dissimilar organisms tend to have similar paths in solving odor-orientation problems (Fig. 1). Crabs and lobsters pursue somewhat meandering upstream paths when stimulated by appropriate odors (Fig. 1A, B). A male moth orienting to a calling female or a synthetic sex-pheromone source (Fig. 1C), a procellariiform bird homing in on a potential source of food, and a salmon orienting to its native stream all must move against the mean direction of fluid flow, and all make turns during their upstream progress (the latter two examples are illustrated in fig. 1 from Arbas *et al.*, 1993). This observation is based upon the similarity of the tracks that the animals follow through space as they navigate toward an odor source, although we must bear in mind the important distinction between those creatures that fly or swim freely through their fluid surroundings (moth, fish, bird) and those that orient while in contact with the substrate (crab, lobster). Even for animals in similar situations, we cannot assume that the behavioral mechanisms that underlie the production of their tracks are the same, even though this assumption is a tempting one. Instead each organism must be viewed within the context of the particular conditions under which the behavior occurs naturally, because these are likely to represent the pressures that have shaped the behavioral and transduction/processing mechanisms over evolutionary time-scales.

Nevertheless, in each of the examples above it seems possible that the fluid environment provides either visual or mechanical stimuli that might be integrated with odor information to provide some measure of the direction of flow. The upwind flight of male moths under the olfactory influence of female-emitted pheromone has been particularly well-studied and extensively reviewed recently (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Kaissling,
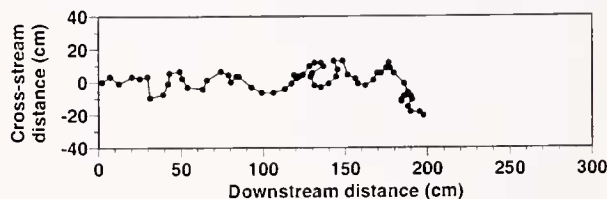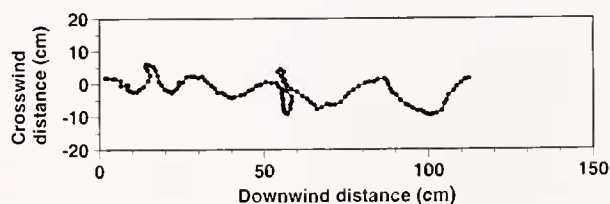

Figure 1. Different organisms frequently have similar paths through space during odor-mediated orientation. However, the similarity in trajectories does not necessarily mean that each animal is employing the same orientation mechanism. (A) A crab, *Callinectes sapidus*, orienting toward an odor source created by a clam in a tidal creek. Flow velocity is 10 cm/s, and the crab's position is marked at 1-s intervals (from Zimmer-Faust *et al.*, 1995, with permission). The boundaries of the plume as visualized by fluorescein dye are indicated by the shaded area. The crab is thought to use a combination of rheotactic and chemotactic orientation mechanisms. (B) The lobster, *Homarus americanus*, orienting to an odor source of homogenized mussel in an experimental flume. Flow velocity was approximately 1 cm/s, and the lobster's position is marked at 1-s intervals (from Moore *et al.*, 1991b, with permission). The results of this study suggested that lobsters used a chemotropotactic orientation mechanism. (C) Flight track of a male moth, *Heliothis virescens*, responding to a point-source of female sex-pheromone. The wind speed was 60 cm/s. Note that the position of the moth is marked every 1/30th s, as compared to the crab and lobster tracks. Male moths have been shown to use a visually guided mechanism to compensate for wind-induced drift and steer upwind (optomotor anemotaxis). The reversals from one side of the wind line to the other that occur along the track are the result of a second program of internally generated turns known as counterturning. Both anemotactic and counterturning mechanisms are modulated by contact with odor. In all three situations, the direction of fluid flow is from left to right (as indicated in A). Note that the scales for A and B are the same, whereas C is enlarged. In A and B, the odor source was located at the graph origin ($X = 0$, $Y = 0$), whereas in C the pheromone source was located about 30 cm further upwind, to the left, of the graph origin.

1997; Willis and Arbas, 1997; Vickers, 1999). Thus an extensive review of olfactory-mediated flight in moths seems unnecessary here. Only relatively specific comments will be made, while integrating the moth system with those of other animals that orient with respect to turbulent, inter-

mittent odor plumes to point out common features of the navigational mechanisms.

Integrating flow and odor detection

As was mentioned earlier, the most reliable method for animals that actively fly or swim to detect direction of fluid flow is to reference movement relative to stationary objects in the environment. This has long been known to occur in insects, as Kennedy (1939) first demonstrated with mosquitoes flying in a plume constituted of his own exhaled breath. Much subsequent work on flying male moths orienting in a pheromone plume has shown that these animals are extremely sensitive to movements of the ground pattern (for example, see Baker and Vickers, 1994). Essentially, the fluid movement causes a discrepancy between the animal's direction of movement (heading or course) in the fluid and static objects in the environment. Resolution of the resultant drift is hypothesized to enable moths to steer more or less into the wind and also to ascertain whether they are making progress against the wind or are "losing ground," i.e., being carried downwind (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Kaissling, 1997; Willis and Arbas, 1997; Vickers, 1999).

The evidence for such visually steered mechanisms in other animals is not yet as convincing, largely because the data derive from observational rather than manipulative, experimental studies. Observations of the flight trajectories and behavior of procellariiform birds over the open sea has shown that they approach sources of odor produced by slicks from downwind, presumably using their extremely well-developed olfactory systems (Nevitt, 1999). Perhaps some sort of visual feedback is utilized to determine wind direction. However, it is not clear how pelagic birds integrate visual cues with olfactory signals, given that the entire ocean is heaving and swelling and otherwise certainly not stationary. The possibility exists that these birds exploit some other mechanism to determine wind direction. The slick odor sources used in such experiments must be essentially invisible to an approaching bird unless other individuals have already arrived at the site, thereby providing visual cues themselves. Thus, the birds that first arrive are presumably the ones most capable of locating the odor source through olfactory signals alone or through olfaction integrated with visual/mechanical cues, and future studies should focus on those species. In other field studies, petrels and shearwaters were observed to approach food sources, nesting colonies, and individual nesting sites under cover of darkness and always from downwind (Grubb, 1972, 1974, 1979). Under such conditions, darkness may preclude the use of visual feedback for the purposes of determining wind-induced drift, again raising the possibility that other mechanisms are employed.

In many species of fish, odor-modulated navigation also appears to occur with the animal moving up-current. However, in only one species has experimental evidence been accumulated that indicates that up-current movement in the presence of odor relies upon visual feedback from objects in the environment (Emanuel and Dodson, 1979). Sharks are also able to locate sources of odor, perhaps by using a combination of rheotactic (an oriented response with respect to the direction of water flow) and chemotactic mechanisms (Hodgson and Mathewson, 1971).

Turning behaviors

Another common feature of the tracks of animals that fly, swim, or walk to odor sources is that up-current progress rarely occurs in directly straight lines (Fig. 1). Most animals observed thus far tend to switch back and forth across the direction of fluid flow. In flying moths, this phenomenon has been well-studied and the mechanism underlying it debated (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Willis and Arbas, 1997; Witzgall, 1997). A number of experiments, which have been summarized in greater detail (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Vickers, 1999), suggest that the species-specific turning rates are driven by an internal "clock" that results in regular reversals from one side of the wind line to the other as the moth makes net progress toward the source. The mechanism, known as counterturning, is integrated with the visually steered compensation for wind-induced drift; together these mechanisms produce the upwind zigzagging flight tracks typical of many insects flying to an odor source (Fig. 1C). Other animals such as birds (Arbas et al., 1993) perform similar maneuvers during upwind flight in response to odor, but again, the actual mechanisms underlying the behavior have not been examined under rigorous experimental conditions. Likewise, fish such as salmon were observed to zigzag as they returned to their home streams (Johnsen and Hasler, 1980).

Substrate-dwelling animals that inhabit aquatic environments have been the focus of many studies upon odor-mediated behavior. Fluid dynamics are somewhat easier to measure and manipulate in the aquatic realm, and so these studies are particularly enlightening as to the relationship between fluids, odors, and the potential mechanisms employed by animals to locate odor sources. Both crabs and lobsters have been the subjects of intensive investigation (Weissburg and Zimmer-Faust, 1993, 1994; Zimmer-Faust et al., 1995; Moore et al., 1991b). For the blue crab, Callinectes sapidus, flow is an important cue in determining successful orientation to an odor source. No-flow or high-flow conditions resulted in poor orientation responses, whereas a slow flow speed resulted in high levels of source location (Weissburg and Zimmer-Faust, 1993). These experiments suggested that males made orientation responses with respect to the direction of flow (rheotaxis). In addition,

these crabs also used chemotaxis, as evidenced by their turning behavior upon crossing the relatively sharp lateral edges of the plume. The turning behavior of *C. sapidus* is therefore distinct from that of flying moths, which respond to an internal turning clock rather than to the detection of a lateral chemical gradient. The lobster *Homarus americanus* also responded to odor plumes but appeared to rely more upon the information contained within and between odor pulses for orientation than upon the direction of flow itself (Moore *et al.*, 1991b). Thus, these animals may use simultaneous bilateral comparisons to remain or turn into the plume and sequential comparisons of odor intensity to advance toward the source (Weissburg, 1997).

Recording odor plume dynamics and behavior simultaneously

In general, the study of fluid dynamics in air is somewhat less tractable than in water. One of the ways that our knowledge of orientation mechanisms and odor plume dynamics has been advanced in recent years is by utilizing techniques that can detect odors or odor representatives. Murlis and co-workers used ion generators and detectors to measure odor plume dynamics in terrestrial habitats (Murlis, 1986, 1997; Murlis *et al.*, 1992). These studies have been particularly instructive in establishing the types of odor stimuli that might be encountered by flying insects, for example.

The actual olfactory appendages of insects have also been successfully used to determine odor plume structure and dynamics. The moth antenna is easily detached and can be readily hooked up to electrodes. Electrical activity measured across the antenna is thought to represent the compound activity of the olfactory receptor cells housed in cuticular projections that often cover the antennal surface by the thousand. The resulting electroantennogram (EAG) has been useful in visualizing pheromone plume structure. Baker and Haynes (1989) used EAGs to measure plume structure in the field and pushed an EAG preparation along a wind tunnel toward a pheromone source to simulate the odor plume that a flying male encounters (Baker and Vickers, 1994). An EAG preparation transported by an intact flying male gave an impression of the frequency and magnitude of olfactory stimulation that a male moth encounters as he flies upwind in a pheromone plume emitted by a single, point-source (Vickers and Baker, 1994a). Lobsters transporting an electrochemical microelectrode and "back pack" gave a similar impression of their olfactory world at the olfactory receptor level (Basil and Atema, 1994). These studies are important because they have allowed us to visualize not only the dynamics of the odor plume but also the kinds of information that are being detected by the actual peripheral olfactory sensors and transmitted to the brain for further processing. Moreover, studies of this type

should reveal to what extent odor plume dynamics are altered by locomotory activity or movement of sensory appendages, an important consideration given that most of the measurements of plume structure to date have used stationary detectors.

Orientating in "restricted" sensory environments

Experiments that alter the sensory world of an animal can be informative as to the mechanisms that those animals use. For example, moths forced to fly through a visually depauperate "blank" tube were unable to remain oriented with respect to a pheromone plume compared to situations in which the visual environment was enriched (Vickers and Baker, 1994b). Baker and Keunen (1982) stopped the wind for flying oriental fruit moths, leaving a stationary plume, suspended in the air. Deprived of the wind-induced drift that is thought to be necessary for steering upwind, some males were still able to locate the odor source, suggesting that other mechanisms can be invoked under certain conditions.

Some animals, however, exist in environments that afford only limited sensory feedback. Such situations might pose navigational problems for these creatures. How, for example, might fish or whales orient with respect to chemical signals in mid-ocean where stationary visual reference points are unavailable because everything drifts in the same current? How do deep-sea dwellers orient where visual cues are unavailable due to the lack of illumination? How do pelagic sea birds reference wind direction with respect to the rolling ocean, moving beneath them? What do insects in dark forests or cave-dwelling animals do when chemical signals beckon, but visual references are unavailable to tell them the direction of flow? Presumably, chemical signals still have a role to play in all these situations. Atema (1995) has suggested that some animals might be able to use "eddy chemotaxis" to orient with respect to chemical cues. In this case, the actual mechanical deformations in the fluid that are associated with the chemical signal are important in guiding locomotory activity. It is certain that mechanical deformation and vortices associated with other organisms can play a significant role in altering the behavior of other organisms. Recently, Yen and co-workers (Doall *et al.*, 1998; Weissburg *et al.*, 1998; Yen *et al.*, 1998) demonstrated that male copepods have the remarkable ability to quite literally track down conspecific females by following their wake structures and possibly the chemical signals contained therein. These tiny planktonic creatures also engaged in "casting" behavior when the mechanical/chemical trail went cold. There are other senses too, that may conceivably be integrated with chemoreceptive input to provide a sense of direction toward an odor source. For example, electric fish are able to "see" their environment: maybe they can use this sense to resolve deformations of the fluid world that they inhabit and couple this information with chemical input to

orient toward an odor source. The lateral line of fish may provide mechanical cues associated with fluid conditions. Perhaps animals can "visualize" fluid flow by means of sonar or thermal sensory systems, particularly in the aquatic realm. In short, any sensory modality that can provide an indication of some attribute of the fluid environment is likely to have been exploited in one way or another. At present our search image is somewhat biased by those sensory modalities that we are most attuned to, namely vision and sound, but animals never cease to amaze us by either the extraordinary heights to which some of their senses are adapted or the existence of "sixth" senses that we simply do not possess.

Changing strategies as a result of moving in and out of odor—or how to span odor gaps?

Intermittency is a feature of any odor plume that is generated in a turbulent fluid environment (Murlis, 1986, 1997; Murlis *et al.*, 1992; Moore and Atema, 1991; Zimmer-Faust *et al.*, 1995; Finelli *et al.*, 1999). By moving in and out of turbulent plumes, organisms are likely to increase intermittency, producing even greater fluctuations in the chemical signal. From an animal's perspective, an important issue revolves around the decision of when to alter behavior in response to odor onset and loss (or perceived loss). Failure to act appropriately may reduce the chances of a timely arrival at a source of odor. On the other hand, some gaps can probably be ignored because they do not represent relevant changes in the stimulus and responding to them would reduce search efficiency. Thus animals must be able to determine how quickly to respond to gaps in odor and what kinds of changes in stimulus parameters (*e.g.*, frequency or concentration) or shifts in flow environment must elicit a response and what kinds can be ignored. The effects of the pulsatile structure of odor plumes upon moth flight behavior have been investigated. Vickers and Baker (1992) determined that male *H. virescens* required the generation of a certain number of pulses per second in order to sustain upwind flight. At a production frequency of fewer than four filaments per second, males were unable to locate the odor source. Subsequent studies revealed that males are actually capable of responding to encounters with individual filaments of odor and, after intercepting an odor filament, they make brief upwind surges towards the source (Vickers and Baker, 1994a, 1996). Other experiments using males of a distantly related moth species, *Cadra cautella*, revealed that this species also responds to encounters with single strands of pheromone (Mafra-Neto and Cardé, 1994, 1996). In both species, the frequency of filament generation has a significant effect upon the directness of upwind flight: the faster the frequency, the straighter the flight. Measurements on *H. virescens* revealed that both the anemotactic component of upwind flight and the counterturning were affected by the

number of odor pulses available (Vickers and Baker, 1996; Vickers, 1999). Thus both mechanisms are directly modulated by odor encounters. These findings have been reviewed extensively recently (Baker and Vickers, 1997; Cardé and Mafra-Neto, 1997; Vickers, 1999).

Relatively little is known about how other organisms respond to odor gaps, or even about how long a time between odor stimuli is necessary to trigger the adoption of a new strategy. A simple strategy to handle gaps in odor stimulation is to arrest upstream movement and wait for olfactory stimulation to resume. This would seem to be particularly advantageous when the flow is relatively steady (producing plumes that do not meander too much), the organism is a substrate dweller, and the target emitting the signal is relatively sessile. Thus, a blue crab tracking the plume emitted by a sedentary mussel is a good example (Zimmer-Faust *et al.*, 1995). Moths do not switch to a new mechanism but adjust both anemotactic and counterturning programs to result in tracks that move side-to-side perpendicular to the direction of mean flow. This behavior, known as casting, is viewed as a way for the animal to resume contact with the odor plume. Casting in *H. virescens* involves increased vertical as well as lateral excursions, thereby facilitating a three-dimensional search of space (Vickers and Baker, 1996). Under certain circumstances, the animals will eventually drift back downwind; videorecordings of oriental fruit moth tracks in the field have shown that, on occasion, these insects will actually pursue a downwind course, perhaps in a effort to regain the odor plume (Baker and Haynes, 1997). Once contact is re-established, the insect may resume upwind flight, trying again to locate the source.

Future Directions

Clearly, there are relatively few systems in which the relationship between fluid dynamics, odor plumes, and animal orientation have even begun to be worked out. One can only hope that future endeavors will include organisms of varying scale that inhabit all types of fluid environment. On the basis of the various mechanisms animals employ to locate odor sources, I suggest that the following areas should prove fruitful for future research.

The olfactory environment

Much of our understanding has come from studies in which discrete odor sources have been used as the source of stimulation in relatively simple fluid environments (laboratory flumes and wind tunnels, tidal creeks and open fields). The actual environments organisms inhabit are considerably more complex in olfactory content and fluid dynamic properties. For example, the trajectories and structure of pheromone plumes created in a forest are different from those in an open field (Elkinton *et al.*, 1987; Willis *et al.*, 1994).

How are an animal's orientation responses modulated in more complex fluid mechanical situations? Are novel mechanisms employed? Understanding how the cues available in such landscapes are sorted, discriminated, and processed will provide insight into the behavioral mechanisms that animals use in their habitat.

Behavioral capabilities in natural arenas

We still know very little about the behavioral capabilities of animals. How far away from an odor source, for example, will a male moth orient, and how do the behavioral mechanisms change as the male approaches the source? Recent field experiments have shown that males generate faster airspeeds and counterturn less frequently farther away from a pheromone source (9–11 m) (Vickers and Baker, 1997). For practical reasons, laboratory studies in wind tunnels or flumes examine the behavior at 1–2 m from the source. How do the fluid dynamics affect the distribution and shape of odor pulses at greater spatial and temporal scales? What are the spatial, temporal, and fluid dynamical limits of behavioral responsiveness? These questions remain largely unanswered.

Odor plume dynamics

Locomotion. It is clear that the locomotor tactics of animals can change the perceived structure of an odor plume. Stationary measures of odor plumes have been and will continue to be instructive. However, an animal must move in order to locate an odor source. To what extent are stationary measurements of odor plume structure affected by introducing movement? Do two moths experience the same kinds of odor pulse fluctuations if one is 1 m away from an odor source and the other is 20 m away, flying twice as fast, and turning less often?

Neurobiology. What features of odor plume structure are detected and represented by peripheral and central nervous systems? Some studies have used neurobiological preparations such as EAGs (Baker and Haynes, 1989; Vickers and Baker, 1994a) or receptor neuron recordings (Baker *et al.*, 1988; Van der Pers and Minks, 1993) to track peripheral events associated directly with actual, behaviorally relevant odor fluctuations instead of with odor tracers or ions. Further experiments that combine neurobiological measurements of actual odor with detection of electrochemical or ion tracers (*e.g.*, Murlis *et al.*, 1990) could be extremely informative in determining those features of filamentous odor plumes that are extracted by the nervous system.

These suggestions are, of course, not exhaustive, but they do seem attainable in the foreseeable future. In conclusion, the importance of fluid dynamics in distributing odors in time and space has clearly been demonstrated in the few systems that have been thoroughly studied. A growing number of observational and experimental reports have strength-ened the notion that animals are efficient at locating sources of odor in their habitats. Their orientation responses are appropriate for the types of odor plume that they typically encounter. Our current understanding of a limited number of model systems in relatively simple settings provides a solid foundation for future endeavors to understand the vital role that chemical signals play in mediating many of life's interactions.

Acknowledgments

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